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Association of polymorphisms in HCN4 with a broad mood-anxiety disorder phenotype

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Abstract

Hyperpolarization activated cyclic nucleotide-gated (HCN) potassium channels are implicated in the control of neuronal excitability and are expressed widely in the brain. HCN4 is expressed in brain regions relevant to mood and anxiety disorders including specific thalamic nuclei, the basolateral amygdala, and the midbrain dopamine system. We therefore examined the association of *HCN4* with a group of mood and anxiety disorders. We genotyped nine tag SNPs in the *HCN4* gene using Sequenom iPLEX Gold technology in 285 Caucasian patients with DSM-IV mood disorders and/or obsessive compulsive disorder and 384 Caucasian controls. *HCN4* polymorphisms were analyzed using single marker and haplotype-based association methods. Three SNPs showed nominal association in our population (rs12905211, rs3859014, rs498005). SNP rs12905211 maintained significance after Bonferroni correction, with allele T and haplotype CTC overrepresented in cases. These findings suggest *HCN4* as a genetic susceptibility factor for mood and anxiety disorders; however, these results will require replication using a larger sample.

Keywords

HCN4 gene; Thalamocortical; Depression; Basolateral Amygdala; Prefrontal cortex; Obsessive-compulsive disorder

1. Introduction

Psychiatric disorders arise through the interplay of genetic and environmental risk factors [17]. Mood and anxiety disorders are highly comorbid [2, 7, 25, 49] and show substantial shared genetic variance based on twin and family studies [11, 23, 24, 31, 36, 51]. Therefore, there are likely to be genetic risk factors that determine risk for both classes of disorders jointly.

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Hyperpolarization activated cyclic nucleotide gated (HCN) ion channels are ion channels that underlie the hyperpolarization-activated current, Ih. HCN channels, coded by HCN 1–4, are composed of four channel subunits [9] and modulate intrinsic neuronal excitability and synaptic integration [12, 31–33, 52]. The open probability of these channels is increased by cyclic adenosine monophosphate (cAMP) [4, 9, 19], making these channels highly susceptible to regulation by receptors coupled to cAMP. Of the four cloned HCN subunits, HCN4 is the most sensitive to cAMP [8, 9].

There are numerous reasons to believe that HCN4 may be involved in mood and anxiety disorders. It has a key role in regulating the functioning of the thalamus, amygdala, mid-brain dopamine system, and indirectly the prefrontal cortex. HCN4 is highly expressed in the thalamus, including the paraventricular nucleus (PVT) [38], the ventrobasal complex, and the reticular thalamic nucleus (RTN) [1]. Lesions in thalamic nuclei induce symptoms of prefrontal cortical (PFC) dysfunction, including impairment of executive function, initiative, and attention [52], suggesting the thalamic nuclei and their cortical fields can act as functional units. Abnormalities in thalamic regions have been described in mood disorders [10, 22] and OCD [18, 20] based on *post-mortem* [5, 58] and *in vivo* anatomical and functional imaging techniques [14, 15]. Orexin inhibits HCN currents [29] and produces anxiety-like responses in rats when injected in to the PVT whereas inhibition of orexin attenuates anxiety [30, 43, 45, 53]. HCN4 channels are highly expressed in the basolateral amygdala (BLA) [38] and channel block in the BLA causes anxiety [44]. HCN channels also play important roles in the functional modulation of the midbrain dopamine (DA) system [12, 37, 41] which has been implicated in depression and other mood disorders [39].

Because HCN4 channels may regulate mood and anxiety by affecting the function of the thalamus, amygdala, and midbrain DA systems, and may indirectly influence PFC function, *HCN4* is a good candidate gene for mood and anxiety disorders. We therefore examined *HCN4* genotype in patients with several different mood and anxiety disorders, including MDD, bipolar disorder, and OCD, and tested for association with a compound mood-anxiety disorder phenotype comprised of these disorders. The positive association findings described here are consistent with a role for HCN4 in mood and anxiety disorders and motivates future research into the role of genetically determined prefrontal connectivity in these disorders.

2. Material and Methods

2.1 Subjects

Variation in *HCN4* on chromosome 15 was characterized in 285 Caucasian patients (mean age = 43.4 ± 11.9 years and 35% male) and 354 Caucasian controls (mean age 61.0 ± 17.9 years and 43% male). The patients included in this study met DSM-IV criteria for mood and/or anxiety disorders as assessed using the Structured Clinical Interview for DSM Disorders (SCID-RV), and included 43 patients with bipolar disorder, 84 with obsessive-compulsive disorder, and 174 with major depressive disorder. Among the bipolar subjects, 11 had a co-morbid anxiety disorder, and among the major depressive disorder patients 20 had a co-morbid anxiety disorder. A total of 13 of the obsessive compulsive disorder cases had co-morbid major depressive disorder. The case phenotype was scored as *Present* if a subject was found to have major depression, bipolar I, bipolar II, and/or obsessive-compulsive disorder. Both healthy controls and patients were recruited via radio advertisement, study flyers and the internet. Patients and controls were assessed using the SCID-RV. Controls had no current or past DSM-IV diagnoses apart from possible nicotine abuse. A standard informed consent was obtained from all subjects. This work was approved by the Yale University Human Investigation Committee.

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2.2 Genotyping

We selected nine tag SNPs in *HCN4* using Haploview software (www.broad.mit.edu/mpg/haploview/) with the Tag SNP Picker routine and Hapmap data to cover all 38.9 kb of the *HCN4* gene. These SNPs met the criteria of being in Hardy-Weinberg equilibrium in the HapMap sample (*P* value ≥ 0.05), an r^2 threshold ≥ 0.8 and minimum allele frequency of ≥7.7% based on Hapmap data (http://hapmap.ncbi.nlm.nih.gov/). Additional SNPs were not included to minimize multiple testing. SNP genotypes were obtained using Sequenom iPLEX Gold on a Sequenom MassARRAY system maintained by the Yale Keck Center. All primer sequences are available upon request.

2.3 Statistical Analysis

Analyses were conducted using the *SNPassoc*, *genetics*, and *haplo.stats* packages in 'R' (cran.r-project.org). The reported *P* values correspond to log-additive models. All analyses included age and sex as covariates. For the analysis of the linkage disequilibrium (LD) pattern and haplotype block delineation we used Haploview. We corrected *P* values using Bonferroni correction for multiple testing as well as using the *Q*-value package in R (http://cran.r-project.org/web/packages/qvalue/index.html). We also calculated sample sizes (samples per group) required for power = 0.8 with alpha = 0.05 based on the observed effect sizes by simulation in R for the non-significant single marker analyses (rs546564 (*n* = 9083), rs548525 (*n* = 2344), rs8030574 (*n* = 1451), rs2623997 (*n* = 662), rs4776632 (*n* = 619), and rs3784812 (*n* = 2966)).

3. Results

All SNPs were in Hardy-Weinberg equilibrium (HWE) in controls. In patients, SNP rs3859014 was not in HWE and SNP rs12905211 had a *P* value of borderline significance (see Table 1), suggesting the possibility that these variants influence disease risk [26]. We found evidence for nominal association between three SNPs (rs498005, rs3859014 and rs12905211) and this group of mood and anxiety disorders (*P* = 0.033, 0.047 and 0.004, respectively). SNP rs12905211 maintained significance after Bonferroni correction (*P* = 0.035) with the T allele being more frequent (OR = 1.5; 95% CI = 1.13–1.98; see Table 1) in cases compared to controls. SNPs rs498005 and rs3859014 did not maintain significance after Bonferroni correction (*P* = 0.297 and *P* = 0.423, respectively). Putative LD blocks were identified. Block 2, including SNPs rs548525, rs12905211 and rs8030574, had two significant associated haplotypes, haplotype CTC, with *P* = 0.004 and GTA, *P* = 0.02, but only the former was significant after Bonferroni correction (OR = 2.88; 95% CI = 1.41–5.90), and haplotype CGT in Block 1 approached significance (see Table 2).

Although power was limited for these analyses we also conducted single marker association analyses for the individual subgroups. Major depressive disorder was significantly associated with two SNPs (rs3859014 with *P* = 0.02 and rs12905212 with *P* = 0.04; see Table 3), whereas anxiety disorders (88 OCD cases) approached significance for association with the SNP rs12905212 (see Table 3).

All analyses included age as a covariate because risk for the phenotype was lower in the older subjects ($p<0.0001$) in our sample. All statistically significant results remained significant when age and sex were not included as covariates.

4. Discussion

In this study, we found an association between rs498005, rs3859014, and rs12905211 in *HCN4* and a group of mood and anxiety disorders; rs12905211 survived Bonferroni correction. Two of the SNPs (rs3859014 and rs12905211) were also significantly associated

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with the MDD subgroup. It is as yet unclear whether these polymorphisms, which are intronic, are causal. We suspect that they are in linkage disequilibrium with a causal variant not included in the study that influences the expression of HCN4 that leads to alterations of cortical-thalamic circuits, amygdala reactivity, and midbrain dopaminergic transmission, and potentially impacting on PFC functioning.

As reviewed above, convergent evidence has implicated HCN channels in the modulation of corticol-subcortical circuitry [27–29, 35, 41, 43, 44, 48, 55, 59] and these circuits are implicated in both mood and anxiety disorders [16, 46]. Our findings with regard to association between SNPs in HCN4 and mood and anxiety disorders is consistent with the known role of HCN4 channels in these circuits, but further research will be required to directly establish the validity of this proposed mechanism. As noted above, blockade of HCN channels in the BLA increases anxiety [44]. It is therefore possible that the variants identified in this study, particularly the T allele of rs12905211, are associated with decreased HCN4 channel expression compared to the C allele.

Given that HCN4 is most dramatically depolarized by the presence of cAMP, altered expression of HCN4 could also potentially impair how stress is modulated since several stress-activated intracellular signaling pathways converge upon cAMP production. It's been shown that the activation of HCN channels on PFC pyramidal neurons weakens the functional connectivity of PFC networks [3, 55]. Studies have also shown that stress, in animal models of depression, potently activates VTA DA neurons [21, 40, 57], which, in turn, stimulate their cortical and limbic targets. Chronic exposure to stress has been shown to cause pathological adaptation in the reward pathway, and this adaptation could contribute to behavioral abnormalities seen in depression and other mood disorders [39]. Therefore, HCN4 may play a role in the dysregulated dopaminergic state underlying the emotional and motivational component of anxiety and mood disorders.

Circadian rhythm misalignment and sleep disturbances are associated with mood disorders [50]. Light sensitive physiological rhythms are controlled by the suprachiasmatic nuclei (SCN) in the hypothalamus. The pacemaker centers of the SCN receive inputs from serotoninergic neurons which regulate the stress response as well as neuroimmunological functions. Agomelatine is a melatonin receptor agonist and 5-HT2C antagonist with antidepressant effects and targets the desynchronised circadian rhythm in mood disorders [42]. M1 cells are the major source of retinal input to SCN [6]. A recent paper has shown that the I_h current in M1 cells is mainly carried by HCN4 channels [54]. Whether HCN4 channels in M1 cells affect mood is unclear at present. However, M1 cells are tightly modulated by dopaminergic innervation [47], and HCN4 channel genetic variation could affect these modulatory influences.

To our knowledge, this is the first reported association between *HCN4* and risk for psychiatric disease. Although this was a small study, the fact that the rs129052111 finding survived Bonferroni correction is promising. Our findings will require confirmation in a larger sample. In addition, exploring the ability of *HCN4*genotype to predict anxiety disorders other than obsessive compulsive disorder, and examining *HCN4*genotype in a larger sample of bipolar patients, will be important in future studies.

5. Conclusion

In conclusion, our results show the first genetic evidence that variation in *HCN4* may be a risk factor for mood and anxiety disorders.

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Highlights

- *HCN4* is a strong candidate gene for mood and anxiety disorders.
- We genotyped nine tag SNPs in the *HCN4* gene in controls and patients.
- Patients had mood disorders and/or obsessive compulsive disorder.
- Three SNPs showed nominal association in our population (rs12905211, rs3859014, rs498005).
- SNP rs12905211 maintained significance after Bonferroni correction.

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Table 1

Single marker association analysis.

SNP/position	N	Minor	%	P	Bonf.	Q-Value	OR/95% CI		Genotypes		PHWE
Block 1								C/C	C/T	T/T	
rs498005/73620310	Case (259)	C	49	0.033	0.297	0.069	1.34/	21.2	55.6	23.2	0.08
	Control (337)		43.9				1.02–1.76	20.2	47.5	32.3	0.51
								A/A	A/G	G/G	
rs3859014/73626439	Case (251)	A	35.3	0.047	0.423	0.069	0.74/	7.6	55.4	37	0
	Control (335)		39.6				0.54–0.99	14.3	50.4	35.2	0.36
								G/G	G/T	T/T	
rs546564/73627770	Case (265)	G	39.8	0.477	1	0.236	1.11/	13.2	53.2	33.6	0.09
	Control (341)		38.4				0.84–1.45	14.7	47.5	37.8	1
Block 2								C/C	C/G	G/G	
rs548525/73627871	Case (266)	C	13.9	0.081	0.729	0.072	1.43/	1.1	25.6	73.3	0.44
	Control (341)		12				0.96–2.13	0.9	22.3	76.8	0.45
								T/T	T/C	C/C	
rs12905211/73628168	Case (265)	T	51.5	0.004	0.036	0.018	1.5/	23.4	56.2	20.4	0.05
	Control (345)		42.2				1.13–1.98	17.1	50.1	32.7	0.66
								C/C	C/A	A/A	
rs8030574/73628214	Case (281)	C	26.3	0.076	0.684	0.072	1.31/	6	40.6	53.4	0.54
	Control (345)		23.2				0.97–1.77	5.5	35.4	59.1	0.88
Block 3								A/A	A/G	G/G	
rs2623997/73628714	Case (266)	A	50.8	0.138	1	0.102	1.23/	23.3	54.9	21.8	0.14
	Control (339)		45.4				0.94–1.61	20.9	49	30.1	0.83
								A/A	A/G	G/G	
rs4776632/73632376	Case (260)	A	43.3	0.429	1	0.236	0.9/	17.3	51.9	30.8	0.38
	Control (331)		48.8				0.68–1.18	23.6	50.4	26	0.91
								A/A	A/T	T/T	
rs3784812/73659194	Case (265)	A	9.1	0.3	1	0.19	1.29/	1.1	15.8	83	0.46
	control(306)		7.7				0.8–2.07	0.6	14	85.3	0.69

Abbreviations: P, P-value for SNP association; Bonf., Bonferroni corrected P value; Q-value, false-discovery rate corrected P value; OR, odds ratio; CI, confidence interval; PHEW, P-values for Hardy-Weinberg equilibrium. All association analyses were adjusted for the effects of age and sex.

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Table 2

Haplotype association analysis of HCN4 and a broad anxiety-mood disorder phenotype.

Haplotype	Case	Control	P	Bonf.	Q-Value	OR	95% CI
Block 1	N = 221	N = 330					
CGG *	38.6	37.5					
TAT	34.5	39.1	0.132	0.396	0.021	0.78	0.56–1.08
CGT	10.2	6.4	0.055	0.165	0.017	1.66	0.99–2.77
TGT	15.7	16	0.749	1	0.079	0.94	0.62–1.41
Block 2	N = 250	N = 332					
GCA *	36.9	43.2					
CCA	1.2	3.5	0.934	1	0.924	1.05	0.31–3.63
CCC	3.7	3.4	0.689	1	0.796	1.22	0.47–3.16
GCC	8	7.7	0.127	0.886	0.293	1.67	0.87–3.21
CTA	1.8	2	0.229	1	0.397	2.01	0.65–6.25
CTC	7.2	3.2	0.004	0.028	0.028	2.88	1.41–5.90
GTA	3.5	2.8	0.021	0.144	0.073	1.59	1.07–2.35
GTC	7.4	8.9	0.543	1	0.752	1.22	0.65–2.28
Block 3	N = 255	N = 298					
AGT *	44.6	41					
AAT	4.7	4	0.921	1	0.921	1.04	0.50–2.17
GAA	6.6	6.6	0.821	1	0.921	1.07	0.59–1.93
GAT	31.2	37.9	0.129	0.514	0.516	0.79	0.58–1.07
GGT	10.4	9.3	0.459	1	0.918	0.83	0.51–1.35

Abbreviations: P, P-value for association analysis; Bonf., Bonferroni corrected P values within block; Q-value, false-discovery rate corrected P values within block; OR, odds ratio; CL, confidence interval; * reference haplotypes. All analyses were adjusted for the effects of age and sex. Block 1: rs498005, rs3859014 and rs546564; block 2: rs54852, rs12905211 and rs8030574; block 3: rs2623997, rs4776632 and rs3784812.

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Table 3

Single marker association analysis of selected SNPs in HCN4 in MDD, OCD and bipolar subgroups.

Sub-group	**N (case/control)**	**MDD**				
SNP		P	Bonf.	Q-value	OR	95% CI
rs498005	161/337	0.12	0.36	0.044	1.3	0.99–1.68
rs3859014	159/335	0.02	0.06	0.022	0.68	0.49–0.95
rs12905211	165/345	0.04	0.12	0.022	1.35	1.04–1.80
Sub-group	N (case/control)	Anxiety				
SNP		P	Bonf.	Q-value	OR	95% CI
rs498005	111/337	0.15	0.45	0.167	1.24	0.93–1.69
rs3859014	102/335	0.52	1	0.385	0.91	0.63–1.26
rs12905211	113/345	0.06	0.18	0.133	1.35	1.04–1.80
Sub-group	N (case/control)	Bipolar				
SNP		P	Bonf.	Q-value	OR	95% CI
rs498005	42/337	0.96	1	0.96	1.01	0.64–1.61
rs3859014	42/335	0.93	1	0.96	1.02	0.61–1.65
rs12905211	40/345	0.63	1	0.96	1.12	0.63–1.75

Abbreviations: P, P-value for association analysis; Bonf., Bonferroni corrected P values within subgroup; Q-value, false-discovery rate corrected P values within subgroup; OR, odds ratio; CI, confidence interval. All analyses were adjusted for the effects of age and sex.